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ERRATUM

The Editors of the Bulletin of the Johns Hopkins Hospital regret exceedingly that in the September issue of that journal there appeared as frontispiece a replica of a medallion of Sir William Osler which was not the medallion presented by his friends and associates at the Johns Hopkins Hospital to the Hospital. The Editors are glad to have this opportunity to publish an illustration of the medallion which *was* presented to the Hospital and to offer an apology to the donors as well as to the readers of the *Bulletin* for the error.

THE INACTIVATION OF THE CHICKEN TUMOR VIRUS BY MEANS OF ALUMINIUM COMPOUNDS[1]

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It has been shown in previous communications (Lewis and Andervont, 1927 a and b) that carmine powder is capable of inactivating the chicken sarcoma virus[2] when added to a 1:10 extract of the tumor in amounts not less than 2.5 per cent of the tumor suspension. Since this property of carmine was found not to be dependent upon the dye principle itself, an effort was made to discover whether the aluminium compounds sometimes used in the manufacture of carmine were responsible for the inactivation.

TECHNIQUE

The technique employed was essentially the same as that already described in experiments upon the adsorption of certain viruses by means of particulate matter (1927 b). The tumor extract was prepared by grinding 3 grams of tumor together with glass in a sterile mortar and diluting it with 30 cc. of sterile Locke solution. The suspension obtained in this way was centrifuged to remove the debris and only the resulting supernatant fluid used for experimental purposes. The aluminium salts to be tested were weighed, placed in tubes, stoppered, and Arnoldized. When they were cool, from 2 to 5 cc. of tumor extract were added to each of the salts. Certain of the aluminium salts dissolved in the tumor extract, so that these had to be tested as solutions and, therefore, although their inactivating properties could be tested, it was not possible to determine whether they adsorbed the virus or

[1] Aided by a grant from the Bache Fund.

[2] This strain of chicken tumor was started by means of the tumor powder devised by Murphy (Rous, 1911). The powder was obtained from Dr. James B. Murphy of the Rockefeller Institute.

not. If the salt did not dissolve in the extract, it was again centrifuged; the resulting supernatant fluid was removed; the deposit was washed through 10 cc. of sterile Locke solution and suspended in the same amount of fresh Locke solution as there was of supernatant fluid. After this both the supernatant fluid and the deposit were used for inoculation. Usually, the supernatant fluid and the washed deposit were inoculated into the same animal, the supernatant fluid being injected into one side of the breast and the washed deposit into the other. In some instances a different fowl was used for each solution. A trace of kaolin was added to the extracts containing the dissolved salts and also to the supernatant fluids before they were injected, because Rous, in 1912, had found that the addition of this substance greatly increased the probability of the occurrence of a tumor and the rapidity of its growth, Podwyssozki, in 1910, having shown that the injection into animals of sterile Kieselguhr, without any tumor-producing agent, either subcutaneously or intraperitoneally, produced a granuloma.

The aluminium compounds used in these experiments were the hydroxide, chloride, sulphate, acetate, lactate, monohydrate, and a basic sulphate.[3]

ALUMINIUM HYDROXIDE

The hydroxide was prepared in the laboratory by precipitation from aluminium chloride and sodium hydroxide. This was tested with the extracts from two tumors. It was added to the extract of one tumor in a concentration of 10 per cent and to that of the other in a concentration of 37 per cent. The addition of 37 per cent of aluminium hydroxide to the tumor extract inactivated the virus and both the supernatant fluid and the washed deposit from this suspension failed to bring about any new growth. On the other hand, the addition of only 10 per cent of the aluminium hydroxide did not inactivate the virus, and while the chicken that received the supernatant fluid from the extract containing this concentration of the salt failed to develop a tumor, the one that was inoculated with the washed deposit did. From these experiments it seemed evident that aluminium hydroxide

[3] The aluminium lactate was made for us by Dr. Read of the Sharpe and Dohme Company of Baltimore, Maryland, and the monohydrate and basic sulphate were presented to us by Dr. Frary of the Aluminium Company of America.

not only adsorbed the tumor virus but, when used in sufficient amounts, fixed it so that it remained inactive in the animal.

ALUMINIUM CHLORIDE

The aluminium chloride dissolved in the tumor extract. It was added in amounts equivalent to 10, 5, 2 and 1 per cent. It was tested with the extracts of 4 tumors. All of these solutions except those containing 1 per cent of the salt prevented the growth of a tumor when inoculated into the breast of a fowl. A concentration of only 1 per cent, however, was not enough to inactivate the virus and the chickens that received the solution of tumor extract containing this amount of the aluminium chloride developed small, slow-growing tumors. From these experiments we may conclude that aluminium chloride in concentrations of more than 1 per cent inactivates the chicken tumor virus.

ALUMINIUM SULPHATE

Two kinds of aluminium sulphate were used to test the inactivating properties of this salt. One of these formed an acid solution, while the other formed a more basic solution. The usual aluminium sulphate, that is, the one which formed the acid solution, failed to inactivate the virus when added to the tumor extract in concentrations as high as 10 per cent. The basic sulphate, on the other hand, inactivated the virus when added to the extract in concentrations as low as 1 per cent. From the few experiments so far performed it is impossible to surmise whether this difference in the inactivating power of the two sulphates is due merely to the greater acidity of the solutions containing the one salt or to some other difference in the chemical nature of the compound.

ALUMINIUM LACTATE

The aluminium lactate dissolved in the tumor extract and formed an acid solution. It was tested with the extracts of 6 tumors and was added to them in concentrations of 10, 5, 2.5, 1, 0.5 and 0.25 per cent. All of the chickens injected with extracts containing one per cent or more of aluminium lactate failed to develop a tumor. Smaller amounts than these were tested only once and in this experiment the

chicken received 5 cc. of an extract containing 25 mgm. of the lactate in one side of the breast, and 5 cc. of an extract of the same tumor containing 12.5 mgm. of the lactate in the other. This chicken developed a tumor on each side. The aluminium lactate forms quite as acid a solution as the aluminium sulphate, and it is hoped that if we can obtain a more basic lactate it will prove to have a greater inactivating power, as was the case with the more basic sulphate.

ALUMINIUM ACETATE

The aluminium acetate did not go into solution in the tumor extract. It was used in only one concentration in only one chicken and with the extract of only one tumor. This salt (0.2 gram) was added to 2 cc. of tumor extract in a concentration of 10 per cent. The resulting supernatant fluid was injected into one side of the breast of a chicken and the washed deposit was injected into the other side. This fowl developed a tumor in each side of the breast, which showed that although this amount of aluminium acetate adsorbed part of the virus, it did not hold it in the animal.

ALUMINIUM MONOHYDRATE

The aluminium monohydrate was tested only once and that was as a concentration of 1 per cent in 5 cc. of tumor extract. Although this amount of the salt did not dissolve in the tumor extract, it was not centrifuged but was injected as a suspension. This concentration (1 per cent) of aluminium monohydrate inactivated the tumor virus and the chicken failed to develop a new growth. The test was made with the extract of only one tumor, but this extract was a most active one, and in this set of ten experiments in which it was used to test ten substances in concentration of 1 per cent, it failed to be inactivated by four compounds and produced tumors in four chickens not including the control. One of the tumors which developed in this set followed the injection of 5 cc. of this extract containing 1 per cent of aluminium chloride.

Discussion

Although many different kinds of substances were used by the great number of investigators who studied the adsorption, fixation and

inactivation of toxins, lysins and viruses, only a few of the aluminium compounds were tried and these by only a few observers.

Roux and Yersin (1889) adsorbed diphtheria toxin by means of aluminium chloride. A filtrate of a broth culture of the diphtheria bacillus was dropped into a solution of aluminium chloride, and when the precipitate thus obtained was washed and inoculated into guinea pigs subcutaneously, the animals died within three to four days. Vaillard and Vincent (1890) used aluminium phosphate to adsorb the tetanus toxin. The aluminium phosphate upon which the toxin had been adsorbed was then injected into guinea pigs subcutaneously. The animals died within 30 to 36 hours with typical symptoms of tetanus. These results indicate that the chloride and phosphate of aluminium are unable to fix the toxins of diphtheria or of tetanus to an extent sufficient to render them inactive when inoculated into the tissues of susceptible animals.

Michaelis (1908), in investigations upon the behavior of ferments, used aluminium hydroxide for the adsorption of trypsin and invertin.

Kraus, Eisler and Fukuhara (1909), while studying the properties of various viruses, demonstrated that aluminium hydroxide adsorbed the rabies virus in dilutions of 1:100. But in this case also the virus was not inactivated.

From these investigations, as well as from our own, it is evident that three phenomena played a part in bringing about the results. One of these is the adsorption of the virus by the particles of the substance used; another is the fixation of the virus onto the particle, and the third is some chemical change between the virus and the substance added which prevents the usual behavior of the virus. According to the investigations of Roux and Yersin (1889), the toxin may be adsorbed but not fixed. It may be adsorbed and fixed so that it is held by the particle *in vitro* but not *in vivo;* or it may be adsorbed and when injected into the animal become fixed so that it is held by the particle *in vivo* although not *in vitro.*

Since a number of the aluminium salts dissolve in the tumor extract thus preventing conclusions as to whether they adsorbed the tumor virus, we have not attempted to compare our findings with the results of other investigators.

CONCLUSIONS

In a study of the inactivating properties of six aluminium salts in respect to the chicken-tumor virus it was found that:

1. A 1-per-cent concentration of aluminium lactate in a 1:10 tumor extract solution inactivated the virus. Smaller amounts failed to inactivate it.

2. An amount of aluminium chloride equal to 2.5 per cent of the extract also inactivated the virus, but 1 per cent of this salt was unable to fix the virus to a degree sufficient to bring about complete inactivation.

3. A basic salt of aluminium sulphate was capable of inactivating the virus when employed in a 1-per-cent concentration. A non-basic salt of aluminium sulphate failed to inactivate the virus in concentrations as high as 5 per cent, although a 10-per-cent concentration of the latter salt did inactivate the virus.

4. A 10-per-cent concentration of aluminium hydroxide adsorbed but did not fix the virus. A 37-per-cent concentration inactivated the virus.

5. A 1-per-cent solution of aluminium monohydrate inactivated the virus.

6. A 10-per-cent solution of aluminium acetate partly adsorbed the virus but failed to inactivate it.

REFERENCES

KRAUSE, R., EISLER, V., AND FUKUHARA 1909 Ueber adsorption des filtierbaren virus. *Ztschr. f. Immunitätsforschung*, i, 307.

LEWIS, M. R., AND ANDERVONT, H. B. 1927 The inactivation of the chicken-tumor virus by means of carmine. *Bull. Johns Hopkins Hosp.*, xl, 265.

LEWIS, M. R., AND ANDERVONT, H. B. 1927 The adsorption of certain viruses by means of particulate matter. *Amer. Jour. Hyg.*, vol. 7, No. 4.

MICHAELIS, L., AND EHRENREICH, M. 1908 Die adsorption Analyse der Fermente. *Biochem. Ztschr.*, x, 283.

PODWYSSOZKI, W. 1910 Zur Frage über die formativen Reize. *Beitr. z. path. Anat.*, xlvii, 270.

ROUS, P. 1911 A sarcoma of the fowl transmissible by an agent separable from the tumor cells. *Jour. Exper. Med.*, xiii, 397.

ROUX, E., AND YERSIN, A. 1889 Contribution à l'étude de la diphtérie. *Ann. de l'Inst. Past.*, iii, 273.

VAILLARD AND VINCENT 1890 Sur le poison tétanique. *C. r. Soc. biol.*, xlii, 634.

ON THE BEHAVIOR OF GRANULATING WOUNDS OF THE RABBIT TO VARIOUS TYPES OF INFECTION[1,2]

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Previous investigations by one of us in association with Kemp (1) have shown that when syphilitic virus is deposited upon the moist surface of a granulating wound of the rabbit, following removal of the crust, the virus is capable of survival and multiplication, so that ultimately, after the wound has healed, a characteristic syphilitic lesion develops in the scar. Not only does a granulating area in the rabbit apparently not offer resistance to the syphilitic virus but it seems to constitute a somewhat more favorable site for its survival than even a fresh wound. Repeated use of this fact has been made in this laboratory for the purpose of producing syphilis in the rabbit, and it has been found that this mode of inoculation yields a very high percentage of successful results, although not quite so high as is obtained by the intra-testicular method.

The apparent susceptibility of granulating wounds of the rabbit to infection with *Treponema pallidum* seemed so contrary to the generally accepted view that granulation tissue is resistant to bacterial infection that it seemed worth while to undertake a series of parallel experiments in which fresh and granulating wounds of the rabbit were exposed to infection with other microörganisms. The purpose of this communication is to record the results of a series of experiments in which both fresh and granulating wounds in the rabbit were infected with *Streptococcus erysipelatis*, *Staphylococcus aureus*, and the organism of fowl cholera (*Pasteurella avicida*).

[1] Aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

[2] Received for publication, July 12, 1927.

[3] Jacques Loeb Fellow in Medicine.

HISTORICAL

It has long since become a generally accepted surgical principle that granulating wounds do not offer a suitable portal of entry for the invasion of the body by the commoner types of pathogenic bacteria so long as the surface of the granulating area remains intact.

Thus, in 1895, Welch (2) wrote as follows:

> "The surface of a healthy granulating wound offers great resistance to the invasion of bacteria, almost as much as an intact exposed surface of the body. Slight injuries, however, such as probing, the removal of dressings, and other manipulations, may convert a granulating surface into a fresh wound, with the accompanying dangers of infection."

Billroth (3) was apparently the first to present experimental evidence in support of this view, during the course of his studies on wound infection. He applied infected dressings to granulating wounds on the backs of dogs without the animals showing any evidence of infection, although the same animals were readily infected and in some instances succumbed when fresh wounds were dressed with the same materials. He concluded that the failure of granulating wounds to absorb poison from infected dressings was due to the absence of lymphatics in the granulation tissue.

Later, a somewhat lively controversy developed in Europe over the question of the absorption of substances into the body from the surface of granulating wounds. The problem received particular attention in Russia and the contributions to it have been well reviewed by Afanassieff (4), but for the most part those who participated in this controversy concerned themselves with soluble substances, and comparatively little attention was directed to the absorption of bacteria from the surfaces of granulating wounds, or the reaction produced by inoculating such wounds with pathogenic microörganisms. Dmitrieff (5) was apparently the first to investigate the behavior of fresh and granulating wounds when inoculated with pure cultures of pathogenic bacteria. According to Afanassieff, this investigator, after inoculating fresh and granulating wounds of rats, guinea-pigs, rabbits and dogs with anthrax bacilli and erysipelas streptococci, came to the conclusion that the character of the dressings applied to the wounds determined the course of subsequent events. If the material used for

the dressings possessed good absorptive power the animals were protected against infection, whereas if it possessed little hygroscopic power the animals were readily infected and succumbed for the most part. Apparently Dmitrieff did not investigate the behavior of wounds toward bacterial infection when no dressings were applied.

Experiments by several investigators, particularly Schimmelbusch (6), demonstrated that anthrax bacilli quickly make their way into the body when inoculated into fresh wounds, but the studies of Afanassieff (4) and Noetzel (7), which appeared almost simultaneously, indicated that the reverse holds true when granulating wounds are inoculated. Noetzel found that if granulating wounds were inoculated with anthrax bacilli the animals survived, whereas if fresh wounds were similarly inoculated, they invariably succumbed. Afanassieff, working with anthrax bacilli and "vibrio Metschnikoffii," obtained the same results. Both investigators stressed the importance of not injuring the granulating area, asserting that trauma sufficient to bring about bleeding could apparently render such an area susceptible and bring about general infection. Afanassieff thought his experiments demonstrated that a rapid destruction of the bacteria took place after they had come in contact with the surface of the wound, and that this destruction was brought about by bactericidal properties of the tissue juices poured out on the granulating surface. He ascribed but slight importance to phagocytosis as a part of the mechanism of the removal of the bacteria. Noetzel, on the other hand, was inclined to lay much more stress upon phagocytosis as an important part of the defensive process, although at the same time he regarded mechanical factors, such as the direction of the flow of the tissue juices, as of great importance. Both investigators, however, regarded the intact granulation tissue as constituting an efficient barrier to systemic bacterial infection. Afanassieff claimed to have obtained evidence that exposure of the bacteria to the influence of the tissue juices of a granulating wound diminished the virulence of the organisms, but Noetzel was unable to confirm this finding. Likewise, Noetzel cound not confirm Afanassieff's finding that inoculation of a granulating wound of an animal with anthrax bacilli rendered the animal immune to subsequent infection with the same organism introduced into fresh wounds.

Cobbett and Melsome (8) found that non-specific inflammatory

reactions in the ears of rabbits, produced by the injection of various chemical irritants, protected the animal against infection with streptococci, but not against anthrax bacilli, diphtheria bacilli or pneumococci.

According to these authors Sestini and Bergonzini had shown that granulations withstood infection from various pathogenic micro-organisms.

Levaditi (9) studied war wounds infected with streptococci and found that they differed in the rate at which they became rid of the organisms. He found that wounds that had become streptococcus-free were resistant to a subsequent infection with the same organism. Levaditi considered that this acquired resistance was the expression of a local immunity. The element of specificity cannot be excluded in his experiments. Gay and Morrison (10) have found that the injection of broth into the pleura protects against infection and they attribute this protection to the presence of large number of clasmatocytes. Gratia (11) has demonstrated that the injection of broth into the skin will protect it against staphylococcus infection. It seems clear from all this work that non-specific inflammatory reactions offer a measure of resistance against some kinds of infection, although the experiments of Chesney and Kemp suggested that a quite different relationship obtains in the case of syphilitic infection.

EXPERIMENTAL

In the experiments of Noetzel and of Afanassieff the extent of the resistance offered by the granulating wounds to bacterial infection was measured by the survival or death of the animal. Local reactions could not be utilised for this purpose, since the control animals in which fresh wounds were inoculated did not live long enough to afford a means of comparison. In the previous experiments reported from this laboratory, in which granulating wounds of rabbits were inoculated with virulent syphilitic virus, the behavior of the granulation tissue toward this type of infection was judged by what transpired at the site of inoculation, in other words, by the local reaction. It became necessary, therefore, when selecting a different organism for comparison with *T. pallidum*, to choose one which would produce a local reaction that would be unmistakable clinically and yet not be apt to

kill the animal. For such a reason *Streptococcus erysipelatis* was chosen and we are indebted to Dr. Harold L. Amoss for supplying us with strains of this organism which served the purpose admirably. Experiments were also carried out with *Staphylococcus aureus* and with *Pasteurella avicida*, but neither of these organisms was entirely suitable, since the former produced only a comparatively slight local reaction and the latter none at all.

Animals. Only rabbits were employed in these experiments. All operative procedures were carried out under full ether anesthesia.

Method. The procedure utilised in previous experiments dealing with syphilitic infection was followed in these experiments. A circular portion of the skin of the back, measuring roughly 6 cm. in diameter, was excised in a series of animals and the wound allowed to granulate without dressings of any sort. Care was exercised to avoid exposing the fascia of the muscles of the back. From 11 to 16 days after excision the crust was removed with sterile forceps as carefully as possible, cultures of various portions of the exposed granulating surface were made by means of a platinum loop and the entire area was then inoculated with washings from a 24-hour blood-agar slant or with a portion of a 24-hour broth culture of the particular microorganism under investigation. As a control, a series of animals with freshly prepared wounds was inoculated with the same emulsion of bacteria. The animals were observed with particular reference to the reaction at the site of inoculation. Cultures were made of the inoculated area at varying intervals after inoculation. Animals dying during the experiment were autopsied and cultures made of the heart's blood.

All experiments were performed in duplicate under comparable conditions. The protocol of a single experiment only will be given in order to indicate the method of experimentation and to avoid unnecessary repetition. The results will then be summarised for each particular organism studied.

Experiment 1. Under ether anesthesia an elliptical area of skin, roughly 6 x 4 cm., was excised from the back of each of a series of 4 normal rabbits. The wound was allowed to granulate without dressings and 14 days later the crust was carefully removed with sterile forceps. The exposed surface of granulation tissue was then cultured.

after which it was inoculated with 2 drops of a suspension of *S. erysipelatis* in physiological salt solution obtained by washing the surface of a 24-hour slant. At the same time, to serve as controls, an area of skin was excised from the back of each of another series of 4 rabbits and the fresh wounds thus produced were inoculated with the same bacterial suspension as soon as bleeding had stopped. Cultures were made from each wound on the fourth day after inoculation.

RESULTS

Streptococcus infection

The results of the experiments conducted in this manner with *Strept. erysipelatis* were clear cut. They are indicated in Table I, from a consideration of which it will be seen that the appearance of the older wounds after inoculation with the erysipelas streptococci was quite different from that presented by the fresh wounds. This difference may be summarised as follows:

a. Old wounds. In only three of the older wounds was there any evidence of local reaction, following inoculation, which might be attributed to the streptococcus infection, and in these it took the form of a slight and relatively transient erythema about the margins of the wound. In the remaining five there was no sign of a fresh local inflammatory reaction. Cultures from these older wounds on the fourth day after inoculation yielded hemolytic streptococci in two instances (in which there was also erythema), and were sterile in the remaining six.

b. Fresh wounds. The appearance of the fresh wounds 48 to 72 hours after inoculation was in marked contrast with that exhibited by the older wounds inoculated at the same time, or by fresh wounds not inoculated with streptococci. Around each fresh wound that had been inoculated there developed a zone of dusky erythema extending 1 to 2.5 cm. from the margins of the wound. In many of the animals there was in addition a boggy edema involving the erythematous area and in some there was actual necrosis of the skin in the inflammatory zone. The more severe reactions persisted for a week or longer. Cultures of these wounds on the fourth day after inoculation showed the presence of hemolytic streptococci in each instance.

TABLE I

Behavior of fresh and old wounds of rabbits toward infection with Strept. erysipelatis

OLD WOUNDS (12–14 DAYS)						FRESH WOUNDS				
Rabbit number	Local reaction after inoculation			Cultures		Rabbit number	Local reaction after inoculation			Cultures after inoculation
	Erythema	Edema	Necrosis	Before inoculation	After inoculation		Erythema	Edema	Necrosis	
							cm.			
1	0	0	0	Sterile	Sterile	9	1	0	0	Hemolytic streptococci
2	Slight	0	0	Gram-positive bacillus	Hemolytic streptococci	10	2	+	0	Hemolytic streptococci
3	0	0	0	Sterile	Sterile	11	2	+	+	Hemolytic streptococci
4	Very slight	0	0	Sterile	Sterile	12	2	+	+	Hemolytic streptococci
5	0	0	0	Sterile	Sterile	13	1	0	0	Hemolytic streptococci
6	0	0	0	Sterile	Sterile	14	2	+	0	Mixed growth, hemolytic streptococci
7	Slight	0	0	Sterile	Hemolytic streptococci	15	2	+	+	Mixed growth, no streptococci
8	0	0	0	Contaminated	Sterile	16	2.5	+	0	Mixed growth, hemolytic streptococci

TABLE II

Behavior of fresh and old wounds of the rabbit toward infection with Staph. aureus

OLD WOUNDS (11–12 DAYS)					FRESH WOUNDS				
Rabbit number	Local reaction after inoculation			Culture after inoculation	Rabbit number	Local reaction after inoculation			Culture after inoculation
	Erythema	Edema	Necrosis			Erythema	Edema	Necrosis	
17	0	0	Small abscess	Staphylococci	24	1.5 cm.	0	0	Staphylococci
18	0	0	0	Staphylococci	25	Slight	0	0	Staphylococci
19	0	0	0	Staphylococci	26	2.5 cm.	0	0	Staphylococci
20	0	0	0	Not done	27	Slight	0	0	Sterile
21	0	0	0	Not done	28	Slight	0	0	Not done
22	0	0	0	Not done	29	Slight	0	0	Not done
23	0	0	0	Not done	30	+	0	0	Not done
					31	+	0	Pus formation	Not done

Staphylococcus infection

Experiments carried out in a similar fashion with *Staphylococcus aureus* were less striking in part because the particular culture employed did not, apparently, possess a high degree of virulence for the rabbit. Nevertheless, a difference was observed between the behavior of the old wounds and that of the fresh wounds toward infection with this organism. The results of these experiments, which are shown in Table II, may be summarised as follows:

a. Old wounds. In none of the old wounds was there any change in appearance 48 to 72 hours after inoculation with *Staphylococcus aureus*, although cultures from three of these wounds on the 4th day after inoculation showed the presence of the organism. In one of these animals a small abscess was found beneath the crust on the 4th day after inoculation.

b. Fresh wounds. In all of the fresh wounds there was clinical evidence of infection 48 to 72 hours after inoculation, consisting for the most part of slight erythema of the surrounding skin. In one animal this erythema extended for a distance of 2.5 cm. from the wound margin and in another a small abscess formed. The local reaction was slightly in excess of that exhibited by control fresh wounds not inoculated with staphylococci. Cultures of three out of four fresh wounds showed the presence of the organism.

Fowl cholera infection

The experiments with *Streptococcus erysipelatis* and *Staphylococcus aureus* seemed to indicate that, under the conditions outlined, granulating wounds of the rabbit are more resistant to infection with these types of bacteria than are fresh wounds, as judged by the appearance of such wounds after inoculation. It seemed advisable to determine whether or not granulating wounds would possess sufficient resistance to protect the animal against infection with an organism which, if it made its way into the circulation, would bring about the death of the animal. For this purpose *Pasteurella avicida* was selected for inoculation. Two experiments similar in character to the others were carried out, parallel series of rabbits with fresh or granulating wounds (11 days) being inoculated with 3 drops of a 24-hour broth culture of this

organism. The results of these experiments are presented in Table III, and may be briefly summarised as follows:

Old wounds. All but one of the 12 animals in which a granulating wound was inoculated with the fowl cholera organism died in from 1 to 8 days, and in all of these the same bacterium was cultivated from the heart's blood.

Fresh wounds. All but 2 of the 12 animals in which fresh wounds were inoculated with the same culture died in from 1 to 3 days, and from all of these *Pasteurella avicida* was recovered from the heart's blood.

TABLE III

Behavior of rabbits with fresh or old wounds after inoculation with Pasteurella avicida

RABBIT NUMBER	OLD WOUNDS	RABBIT NUMBER	FRESH WOUNDS
32	Dead, 24 hours	44	Dead, 48 hours
33	Survived	45	Survived
34	Dead, 24 hours	46	Dead, 3 days
35	Dead, 24 hours	47	Dead, 24 hours
36	Dead, 24 hours	48	Survived
37	Dead, 48 hours	49	Dead, 24 hours
38	Dead, 8 days	50	Dead, 3 days
39	Dead, 4 days	51	Dead, 3 days
40	Dead, 3 days	52	Dead, 24 hours
41	Dead, 24 hours	53	Dead, 3 days
42	Dead, 3 days	54	Dead, 24 hours
43	Dead, 3 days	55	Dead, 3 days

It is clear from these results that under the conditions of the experiment the surface of a granulating wound in a rabbit does not offer an impenetrable barrier to invasion of the blood stream by this particular organism. The experiments with *Streptococcus erysipelatis*, nevertheless, seemed to indicate that wounds that had reached the granulating stage possessed some degree of resistance against infection with that organism, since they reacted in a manner quite different from that exhibited by fresh wounds. In order to ascertain whether or not this difference was due to local effects or was a property of the entire body, a further experiment was carried out in duplicate. Wounds were made on the backs of a series of rabbits in the usual manner and were allowed to granulate. From 12 to 16 days later a second wound

was made on the back of each animal at several centimeters distance from the first, and both wounds were then inoculated with equal amounts of a suspension of *Strept. erysipelatis*. Opportunity was thus afforded to contrast the behavior of fresh and granulating wounds *in the same animal* toward infection with erysipelas streptococci. The results of this experiment are shown in Table IV and may be briefly stated as follows. In none of the old wounds was there the slightest sign of a renewed inflammatory reaction following inoculation, whereas all of the fresh wounds showed extensive erythema of the surrounding skin and in at least half of them there was edema as well. The contrast

TABLE IV

Behavior of old wounds and fresh wounds in the same rabbit toward infection with Strept. erysipelatis

RABBIT NUMBER	OLD WOUNDS (12–16 DAYS)			FRESH WOUNDS		
	Local reaction after inoculation			Local reaction after inoculation		
	Erythema	Edema	Necrosis	Erythema	Edema	Necrosis
56	0	0	0	Slight	0	0
57	0	0	0	+	+	0
58	0	0	0	+	+	0
59	0	0	0	+	+	0
60	0	0	0	+	0	0
61	0	0	0	+	0	0
62	0	0	0	+	+	0
63	0	0	0	+	0	0

was quite striking and indicated that the absence of signs of reaction in granulating wounds following infection with *Strept. erysipelatis* was due to a change in the local terrain and not to any change in the animal as a whole.

The results of bacteriological study of the granulating wounds after inoculation with *Strept. erysipelatis* suggested that these organisms were actually disposed of in some manner, but whether the disposal was accomplished by an active destructive mechanism or whether it was due to the fact that the organisms die because they encountered an unfavorable environment was not disclosed by the experiments.

Several fresh and granulating wound areas were excised 3 to 4 days after being inoculated with streptococci, and sections were made and

stained for bacteria. Study of these indicated that in the granulating wounds the streptococci had scarcely penetrated beneath the surface of the granulation tissue whereas in the fresh wounds they could be found at a considerable distance in the deeper tissues. In like manner the fascia of the muscles beneath the infected fresh wounds was found to exhibit gross evidence of inflammatory reaction, whereas in the case of the granulating wounds there were no gross signs of inflammation of the same structure. The question naturally arose; was there any extra-cellular bactericidal principle operative in the granulating wounds which disposed of the streptococci or limited their invasion? Could such wounds harbor a bacteriophage? Afanassieff, as we have already seen, thought that the tissue juices of granulating wounds possessed bactericidal properties, and recently Bergel (12) has ascribed a similar property to fibrin. In order to obtain information upon this point the following experiment was carried out.

The granulating base of a 14-day wound on the back of each of two normal rabbits was excised under ether anesthesia. The layer of granulation tissue thus obtained, which measured approximately 2 mm. in thickness and roughly 2 × 4 cm. was then ground up in a sterile mortar with 25 cc. of physiological salt solution and filtered through a Berkefeld N filter. The filtrate thus obtained was tested for the presence of a lytic principle for *Streptococcus erysipelatis* in the following manner.

Two tubes of horse-serum broth were inoculated with *Strept. erysipelatis* and immediately thereafter to one was added 2 drops and to the other 20 drops of the filtrate described above. Horse-serum agar slants were then streaked with loopfuls of material from each tube, after which the original broth tubes and the agar slants were incubated at 37°C. After 6 hours' incubation sub-cultures were made from the serum-broth tubes (containing filtrate) on horse-serum agar slants. All the slants were examined for evidence of lysis during the process of incubation.

In addition, to 6-hour broth cultures of *Strept. erysipelatis* were added, in one case 2 drops, and in the other 20 drops of the filtrate, after which the cultures were incubated at 37°C. Sub-cultures from these serum-broth-filtrate tubes were made on serum-agar slants just prior to incubation and after 6 hours at 37°C.

The results of this series of experiments were entirely negative. Not the slightest evidence was obtained that there was present in the extract of the granulation tissue a lytic principle for hemolytic streptococci.

DISCUSSION

It would appear from the foregoing experiments that when, under the conditions specified, a granulating wound in the rabbit was inoculated with a strain of *Streptococcus erysipelatis* which is capable of bringing about an intense inflammatory reaction in a fresh wound, little or no sign of a renewed inflammatory reaction was observed in the surrounding skin area. Furthermore, cultures from such a wound 3 to 4 days after inoculation indicated that the streptococci disappeared or diminished in number. Somewhat less striking were the results obtained when the granulating wounds were inoculated with *Staphylococcus aureus*, but they tended to show essentially the same phenomenon, although the staphylococci did not disappear so rapidly from the granulating surface as did the streptococci. The experiments with these two types of organisms confirm, therefore, the older work of Billroth, Afanassieff, and Noetzel, to which reference has already been made.

The absence of signs of acute inflammatory reaction in the skin surrounding the granulating wounds following inoculation, the frequency with which cultures made from the granulating area were sterile, the absence of bacteria in the deeper layers of the granulation tissue, as revealed by histological study, and the fact that the underlying fascia of the muscles did not show signs of acute inflammation, all tend to indicate that in granulating wounds there is a local mechanism the function of which is to limit infection and prevent invasion of the body by the organisms mentioned. That this mechanism, whatever it may be, does not afford complete protection under the conditions of the experiment, however, is shown by the experiments with the fowl cholera bacillus. This organism was able to make its way through the granulation tissue, invade the circulation, and bring about the death of the animal in almost every instance. Inasmuch as this bacterium possesses such a high degree of virulence for the rabbit, it is conceivable that only a very few organisms might need to make

their way into the circulation and set up a fatal infection. The act of removing the crust from the granulating wound in practically every instance brought about a small amount of bleeding, no matter how much care was exercised, and the fact that all but one of the rabbits succumbed indicates that even such a small amount of trauma was sufficient to permit systemic infection through the granulation tissue.

While, therefore, we may be reasonably sure that after the removal of the crusts from the granulating wounds small atria were opened up, nevertheless the fact remains that when such wounds under identical conditions were sown with streptococci or staphylococci, which of themselves were capable of setting up a local reaction in fresh wounds but were not sufficiently virulent to cause the death of the animal, very little or no fresh local inflammatory reaction was observed in the skin surrounding the granulating area, and in many instances the infecting organisms were apparently completely disposed of. One can scarcely escape the conclusion that in the granulating wounds there was a state of increased resistance against these two types of bacteria. This conclusion is in conformity with long-established surgical principles and with the recent work of Gay (13) and his associates on local immunity to streptococcus infection in serous cavities.

The failure to demonstrate the presence of any lytic principle in extracts from granulation tissue suggests that the resistance factor is not dependent upon a bacteriophage operating *in situ*, and directs attention to the serum bacteriolysins, or the cells, as being the important elements in the defensive mechanism. No attempt has been made by us to study this phase of the subject, since we were concerned not so much with analysing the mechanism of the local defensive process as with trying to determine the behavior of granulating wounds toward types of infection other than syphilitic. Previous work from this laboratory had seemed to indicate that such wounds were highly susceptible, or at any rate not resistant, to infection with *Treponema pallidum*. The experiments reported in this communication indicate that a quite different state of affairs obtains with regard to *Streptococcus erysipelatis* and *Staphylococcus aureus* when identical conditions aremaintained. These latter organisms do not long survive or readily produce a local reaction when inoculated upon granulating

wounds of rabbits, whereas *T. pallidum* can survive in such an area, can multiply and can bring about a characteristic syphilitic lesion.

The explanation of the difference in the reaction of granulating wounds toward syphilitic infection on the one hand, and toward streptococcic and staphylococcic infection on the other, is not by any means clear and had probably better be left for the future to determine. The apparent susceptibility of such wounds to syphilitic infection can scarcely be ascribed to mechanical factors such as defects in epithelium or the opening up of lymph or blood channels through removal of the crust. If such factors were responsible for the susceptibility of granulation tissue to syphilitic infection why do they not operate also in favor of streptococcic or staphylococcic infection, and why are not fresh wounds as susceptible to syphilitic infection as granulating wounds? There are experiments in progress at the present time which are designed to show that the apparent predisposition of granulating wounds to syphilitic infection is not due merely to mechanical factors such as loss of epithelium or opening up of lymph channels through trauma incidental to the removal of the crust, but to other and perhaps more obscure causes. A report upon these experiments will be forthcoming later.

SUMMARY AND CONCLUSIONS

When granulating wounds (11 to 16 days old) of the rabbit, after removal of the crust, were inoculated with cultures of *Streptococcus erysipelatis*, the surrounding skin area in the succeeding days showed little or no sign of an acute inflammatory reaction, whereas when fresh wounds in the same animal were inoculated in an identical manner with the same culture the surrounding skin showed signs of an intense inflammatory reaction which persisted for a number of days. A similar although less marked difference was observed between the behavior of granulating wounds and that of fresh wounds after inoculation with *Staphylococcus aureus*.

Bacteriological study indicated that in the case of the granulating wounds the bacteria quickly disappeared from the surface of the granulating area, but did not do so in the case of the fresh wounds. Histological study failed to reveal the presence of bacteria in the deeper portions of the granulating area, but they could be demonstrated well

below the surface of the fresh wounds. It was not possible to demonstrate in extracts of granulation tissue of the rabbit the presence of any lytic principle similar in its mode of action to bacteriophage.

When granulating wounds of the rabbit were inoculated with broth cultures of *Pasteurella avicida* the organisms were able to make their way into the circulation and kill the animals in nearly every instance, even when the greatest care was exercised not to disturb the granulating area.

Upon the basis of the data afforded by these experiments and by others in which syphilitic virus was used for inoculation, it is concluded that granulation tissue in the rabbit appears to constitute a relatively unfavorable environment for the survival and growth of streptococci and staphylococci, whereas it appears to offer a favorable locus for the survival of the treponemes of syphilis. This difference in behavior of granulation tissue in the rabbit to different kinds of pathogenic microörganisms makes it difficult, if not impossible, to formulate a general principle, applicable alike to all types of bacteria, regarding the susceptibility of granulating wounds to bacterial infection.

REFERENCES

(1) Chesney, A. M., and Kemp, J. E.: *Jour. Exp. Med.*, 1925, xli, 487.
(2) Welch, W. H.: Dennis' System of Surgery, Vol. 1, p. 284, Philadelphia, Lea Bros. and Co., 1895.
(3) Billroth, Th.: *Arch. f. klin. Chir.*, 1865, vi, 443.
(4) Afanassieff, N.: *Beitr. z. Path. Anat. u. z. Allgemein. Path.*, 1897, xxii, 11.
(5) Dmitrieff: Cited by Afanassieff (4).
(6) Schimmelbusch, C.: *Deutsche med. Wchnschr.*, 1894, xx, 575.
(7) Noetzel, W.: *Arch. f. klin. Chir.*, 1897, lv, 543.
(8) Cobbett, L., and Melsome, W. S.: *Cntrlbl f. Allgemein. Path. u. Path. Anat.*, 1898, ix, 827.
(9) Levaditi, C.: *Compt. rend. Soc. de biol.*, 1918, lxxxi, 1059.
(10) Gay, F. P., and Morrison, L. F.: *Jour. Inf. Dis.*, 1923, xxxiii, 338.
(11) Gratia, A.: *Compt. rend. Soc. de biol.*, 1923, lxxxix, 826.
(12) Bergel, S.: *Klin. Wchnschr.*, 1925, iv, 1952.
(13) Gay, F. P.: *Physiol. Reviews*, 1924, iv, 191.

THE RELATION OF THE PERITONEAL MESOTHELIAL CELLS TO THE PRODUCTION OF ASCITES[1]

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When one examines the viscera of an animal which has been made edematous by the intravenous infusion of large amounts of fluid, it is found that certain areas are much more markedly edematous than others. The connective tissue about the pancreas is always heavily loaded with fluid; the liver is markedly swollen, and the intestinal wall, likewise, is generally much thickened. On the other hand, very little fluid seems to collect in the kidneys and the spleen never becomes edematous. In fact, Cunningham[2] found that the spleens of animals which had received enormous amounts of saline intravenously showed no increase in size or proportion of fluid content over those of normal animals. This uneven distribution of fluid is obviously the result of the special structure of the organs concerned, and is probably a connective-tissue affair in all of the organs referred to except the liver where the fluid is largely, if not wholly, within the cells of the parenchyma. The fact that it has been found that more fluid collects in certain tissues than in others suggested that this difference in reaction of the organs bordering it might have some bearing upon the collection of fluid in the peritoneal cavity. That is to say, there seemed to be a possibility that the variable abilities of the tissues, adjacent to the cavity, to store fluid, might have an important bearing on the formation of ascites.

The above general considerations, together with the report by Cunningham (1) that the mesothelial cells covering the spleen became markedly edematous during the collection of fluid in the peritoneal cavity, suggested the possibility that the spleen might represent a route through which fluid could reach the peritoneal cavity more rapidly and more easily than through the other organs covered by

[1] Received for publication, July 20, 1927.

[2] Personal communication.

peritoneum. The first series of experiments to be reported here was designed to test this hypothesis; and from them it was found that splenectomy made no appreciable difference in the rate of collection or amount of eventual ascites, and therefore the possibility that the spleen is especially correlated with ascites was ruled out.

It next seemed wise to investigate the reactions of the liver, since it represents the opposite extreme, in that it increases greatly in size and retains a large amount of fluid when an animal is made edematous. In the case of the liver, the investigations were planned to test whether or not variations in the blood supply would change the amount of fluid escaping into the peritoneal cavity during the intravenous exhibition of fluid. It was found that when either the hepatic artery or portal vein was ligated there was a moderate increase in the amount of ascites. On the other hand, when the same amount of tissue laceration was carried out in the neighborhood of the liver, but without ligation of the vessels, there was an equal increase in the ascites. Therefore it seems obvious that neither the areas in which large amounts of fluid collect, nor those in which little or none is stored, can be considered as especially concerned with the transference of fluid from the blood stream to the peritoneal cavity.

DESCRIPTION OF EXPERIMENTS[3]

Adult cats, male and female, though appearing uniform in size, were found to differ very strikingly in weight, so that throughout these experiments the exact weight of the animal was taken into consideration from a mathematical viewpoint. Our method of tabulation has reconciled the weight with the fluid infused and transuded by using as the ordinates of our curves the percentage relationship between the body-weight in grams and the fluid infused in cubic centimeters; the abscissae representing the percentage ratios between the fluid infused and the fluid present as ascites at the end of each experiment. Particular attention was paid to the weight of the animals in these experiments because it has seemed, from a study of the literature, that some of the inconsistent findings which have been reported may have been due to the lack of attention in regard to this particular point.

[3] The writer thanks Dr. Robert S. Cunningham for the aid given by his counsel and encouragement in the execution of these experiments.

The experiments reported in this paper have been carried out under as uniform conditions as could be obtained. The animals were taken from the stock room, in which they had been kept under quite uniform conditions, and operated upon immediately, so that any variations in the fluid content would be only such as would come within the normal limits of satisfaction of thirst on the part of the individual animal. The animals were anesthetized with ether, weighed and immediately fixed on a small animal table. The long saphenous vein was generally utilized for the injection; the cannulization usually requiring from three to five minutes before the injection was begun. The fluid used was a sodium chloride solution of 0.85 per cent made up in distilled water. The injection fluid was kept at body temperature throughout the experiment and was allowed to flow (at a constant rate) into the vein by hydrostatic pressure during the selected period (60 minutes). At the end of this time the animal was sacrificed and the fluid in the peritoneal and pleural cavities was immediately aspirated and measured.

In the animals of the splenectomized series the preliminary operation was performed from two to three weeks prior to the final experiment and only those animals were used for the infusions in which recovery had been wholly uneventful. Inasmuch as the ligation of the portal vein and of the hepatic artery are both procedures from which either death or extreme pathological changes may result very quickly, it was decided to utilize the animals for the final experiments immediately after the preliminary ligation. Under such conditions it is obvious that the laceration of tissues would permit a certain leakage into the peritoneal cavity. This factor was controlled by a separate series of experiments in which the animals were subjected to no operative procedures except a mild laceration of the connective tissue about the hepatic duct. In all the operations on the liver, the viscera were kept moist by applications of warm gauze soaked in saline, but great precautions were taken to prevent the accumulation of any fluid in the peritoneal cavity from the applications. When the operation was completed, the gauze packs were removed and the cavity carefully inspected to control any oozing points or accidental collections of fluid. The abdominal incision was closed by everting the cut edges of peritoneum, muscle and skin, and holding these approximated by

means of a long intestinal clamp. The operating board was then so tilted that the head was slightly lower than the body of the animal. This position was used in all experiments and was found to be very satisfactory, since it permitted any bronchial secretion to drain without respiratory distress, and reduced the effect of venous pressure on the inflow of saline.

The experimental results are presented in four charts. In each of these charts the findings from a series of normal cats, which were studied

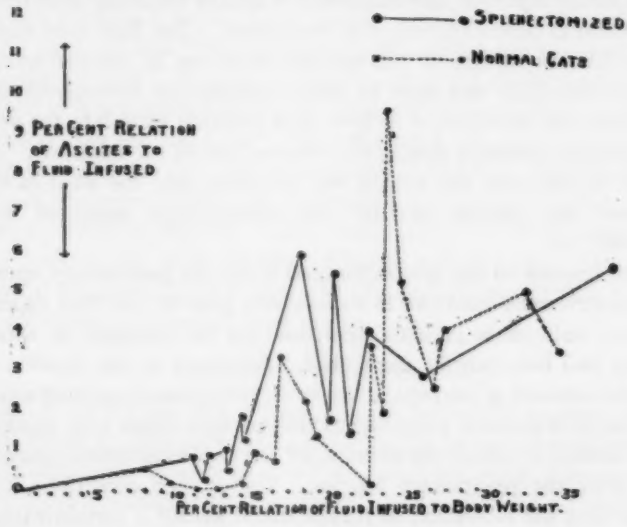
Splenectomized Cats
Normal Cats
Per Cent Relation of Ascites to Fluid Infused
Per Cent Relation of Fluid Infused to Body Weight.

Chart I

experimentally as outlined above, are plotted in a broken line. These normal animals were used as controls for all the experiments, and hence it is obvious that the normal curve appearing on each chart is the same. It will be noted that this group is quite irregular; there being a considerable number of animals which differed quite markedly from the average in their response to the injected fluid. But, on the whole, the amount of fluid found in the peritoneal cavity increased progressively with the amount introduced. In Chart I the results obtained from the series of splenectomized animals are plotted in

TABLE 1

Normal cats

NUMBER	SEX	WEIGHT	FLUID INJECTED	ASCITES PRESENT	RELATION OF FLUID INJECTED TO BODY-WEIGHT	RELATION OF ASCITES TO FLUID INFUSED
		grams	*cc.*	*cc.*	*per cent*	*per cent*
29	M.	3,135	245	1.3	7.8	0.53
37	M.	1,890	200	0.4	9.5	0.20
44	F.	2,140	250	Trace	11.2	Trace
34	M.	2,520	315	Trace	12.5	Trace
32	F.	1,995	280	0.5	14.0	0.18
20	F.	2,160	320	3	14.8	0.94
18	M.	3,010	485	3.5	16.1	0.72
46	M.	1,800	295	10	16.4	3.35
36	M.	2,195	395	9	18.0	2.28
19	F.	1,970	380	5	18.8	1.35
30	F.	1,040	230	0.3	22.1	0.13
12	M.	3,690	830	30	22.2	3.62
26	F.	1,770	405	8	22.9	1.97
22	M.	3,425	798	76	23.2	9.53
35	F.	1,750	420	22	24.0	5.25
24	F.	1,465	385	10	26.2	2.60
21	M.	1,215	324	13	26.8	4.04
23	F.	1,440	460	23	31.9	5.00
27	M.	1,100	375	13	34.1	3.47

TABLE 2

Splenectomized cats

NUMBER	SEX	WEIGHT	FLUID INJECTED	ASCITES PRESENT	RELATION OF FLUID INJECTED TO BODY-WEIGHT	RELATION OF ASCITES TO FLUID INFUSED
		grams	*cc.*	*cc.*	*per cent*	*per cent*
43	F.	2,255	245	2.0	10.8	0.82
50	M.	3,030	385	1.0	11.7	0.26
40	M.	1,920	230	2.0	12.0	0.87
41	F.	2,220	290	3.0	13.1	1.03
38	F.	1,795	235	1.2	13.2	0.51
42	F.	2,665	375	7.0	14.0	1.86
39	F.	1,100	155	2.0	14.1	1.28
55	F.	2,580	455	27.0	17.6	5.94
48	F.	2,305	450	3.5	19.5	1.71
52	F.	1,940	385	21.0	19.8	5.45
51	M.	2,650	560	8.0	21.2	1.43
49	F.	1,030	225	9.0	22.2	4.00
53	M.	1,215	310	9.0	25.5	2.90
54	F.	695	250	14.0	36.0	5.60

black and it is immediately obvious that the amount of fluid appearing in the peritoneal cavity after splenectomy is greater than that in normal animals up to a certain point, after which the two run parallel. That there is a greater amount of fluid appearing in the peritoneal cavity after splenectomy would indicate that not only is the spleen not a specialized area through which the fluid transudes into the peritoneal cavity, but that the amount of ascites forming after splenectomy is even greater than in normal animals. The difference in these two groups of experiments is, however, so very small that it seems legitimate to conclude that the normal and splenectomized animals do not show any important differences. The data for the normal animals are given in Table 1, while Table 2 contains the data obtained from the splenectomized cats.

When we turn to Charts II and III (Tables 3 and 4), which show the amounts of ascites formed when animals are infused after the ligation of the hepatic artery and the portal vein, respectively, a marked increase in the ascites is found in both instances, and this increase becomes apparent much earlier than is the case in either the normal or the splenectomized animals. Chart IV (Table 5) shows the amounts of fluid which collected in the peritoneal cavities of five animals, in which there was no operative procedure other than a mild laceration of the tissues about the portal vein and the common bile-duct. It is obvious that the ascites produced by this procedure is quite as marked as that found after ligation of the hepatic artery or portal vein. In general, then, these experiments do not demonstrate that there are any specific areas bordering on the peritoneal cavity which have any special importance as "portals" of entry through which transudation into the peritoneal cavity may occur.

In all the animals the fluid appearing in the pleural cavities was measured, and in no case was there anything striking in the results obtained, except that the fluid invariably appeared in the right pleural cavity before any was obtainable from the left, and none was ever found in either before a considerable amount of ascites had accumulated. Attempts were made to measure the pericardial fluid, but the amounts were so small that no conclusions could be drawn with reference to any specific differences.

Tissues were saved from all the animals used, and were carefully

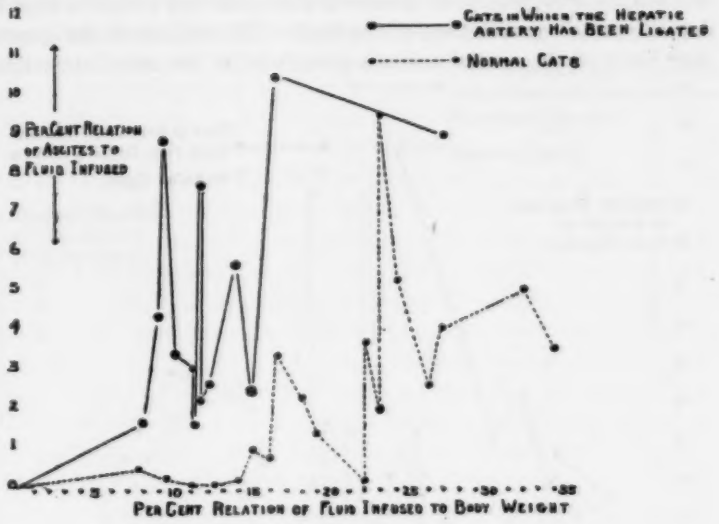
Cats in Which the Hepatic Artery Has Been Ligated
Normal Cats
Per Cent Relation of Ascites to Fluid Infused
Per Cent Relation of Fluid Infused to Body Weight

CHART II

TABLE 3

Cats with hepatic artery ligated

NUMBER	SEX	WEIGHT	FLUID INJECTED	ASCITES PRESENT	RELATION OF FLUID INJECTED TO BODY-WEIGHT	RELATION OF ASCITES TO FLUID INFUSED
		grams	*cc.*	*cc.*	*per cent*	*per cent*
87	M.	2,345	185	3	7.9	1.62
89	F.	2,615	230	10	8.8	4.35
93	M.	2,205	195	17	8.8	8.83
75	F.	3,285	325	11	9.9	3.39
88	M.	1,475	165	5	11.2	3.03
91	F.	2,900	325	5	11.2	1.57
95	F.	1,780	205	4.5	11.5	2.20
86	F.	2,695	300	23	11.5	7.68
77	F.	2,690	325	8.5	12.1	2.62
90	M.	2,080	285	16	13.7	5.62
72	F.	2,510	370	9.0	14.7	2.43
74	M.	1,295	210	22	16.2	10.48
73	F.	1,460	390	35	26.9	8.95

studied histologically to determine if they gave any evidence regarding the transit or collection of the fluid. The sections of the spleens and livers of the normal animals proved to be the most interesting.

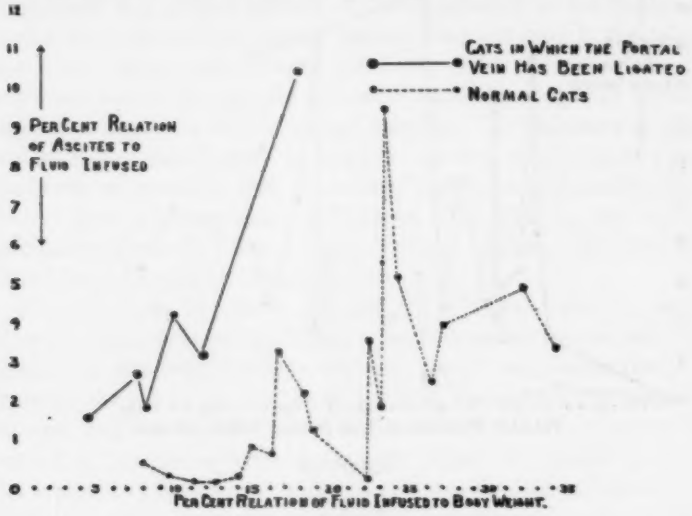
Cats in Which the Portal Vein Has Been Ligated
Normal Cats
Per Cent Relation of Ascites to Fluid Infused
Per Cent Relation of Fluid Infused to Body Weight.

Chart III

TABLE 4

Cats with portal vein ligated

Number	Sex	Weight	Fluid injected	Ascites present	Relation of fluid injected to body-weight	Relation of ascites to fluid infused
		grams	*cc.*	*cc.*	*per cent*	*per cent*
101	M.	3,390	150	2.5	4.4	1.67
104	F.	2,385	180	5.0	7.6	2.78
105	M.	2,880	235	4.5	8.2	1.91
100	F.	2,505	245	10.5	9.8	4.28
102	F.	1,725	200	6.5	11.6	3.25
103	F.	1,635	285	30	17.6	10.53

In most cases, the spleen presented the appearance of a wholly normal organ except that, in those animals which had received considerable amounts of fluid, all the mesothelial cells covering the organ contained

definite droplets of fluid, which in many instances were remarkably large (Figs. 1 and 2). Careful study of many sections of the spleens of

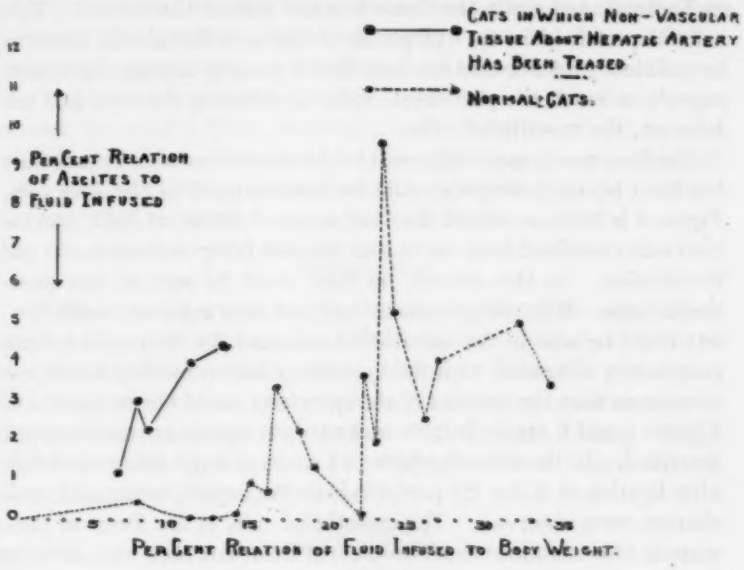
Cats in Which Non-Vascular Tissue About Hepatic Artery Has Been Teased
Normal Cats.
Per Cent Relation of Ascites to Fluid Infused
Per Cent Relation of Fluid Infused to Body Weight.

CHART IV

TABLE 5

Cats in which relatively non-vascular tissue about the hepatic artery has been teased

NUMBER	SEX	WEIGHT	FLUID INJECTED	ASCITES PRESENT	RELATION OF FLUID INJECTED TO BODY-WEIGHT	RELATION OF ASCITES TO FLUID INFUSED
		grams	*cc.*	*cc.*	*per cent*	*per cent*
98	F.	2,845	180	2.0	6.3	1.11
94	M.	2,580	195	6.0	7.6	3.08
92	F.	2,680	215	5.0	8.0	2.32
97	F.	2,355	260	10.5	11.0	4.04
96	F.	2,050	270	12.0	13.2	4.45

these animals indicated very strongly that none of the cells in the pulp, trabeculae or capsule of this organ stored any fluid. Figure 2 shows the capsule and the overlying layer of the mesothelial cells, and it is

obvious that the former is entirely normal, while the latter is markedly edematous. Figure 3 shows the pulp of the same spleen as that shown in Figure 2, and again the tissue is quite normal throughout. This collection of fluid in the cytoplasm of the mesothelial cells seems to be sufficient evidence that the fluid that is passing through the splenic capsule to reach the peritoneal cavity is diffusing through, and not between, the mesothelial cells.

The liver reacts quite differently. In every case where much fluid has been injected, droplets could be demonstrated in the liver cells. Figure 4 is from an animal that had received 380 cc. of fluid, and the liver cells contained from one to four droplets fairly uniform in size and distribution. In this animal, no fluid could be seen in the mesothelial cells. When larger amounts of fluid were injected, small droplets could be seen in the mesothelial cells and the liver cells became enormously distended with fluid; in many instances they became so edematous that the outlines of the cytoplasm could not be made out. Figures 5 and 6 are both from such extreme experiments as those just described. In the animals which had received large amounts of fluid after ligation of either the portal vein or the hepatic artery, the same changes were observed. The mesothelial cells of the livers of these animals also contained small droplets of fluid, but they were never so large as those found in the splenic mesothelium of the same animals.

DISCUSSION

The distribution of fluid in the animal body is a subject which has received a great amount of attention; and the fact that in certain clinical entities there is a tendency for fluid to collect in the serous cavities is well known. Many suggestions have been offered to explain these localized collections of fluid, all of which have been discussed in detail in the recent reviews of Loeb (2) and of Rowntree (3). The present series of experiments were begun with the idea in view that perhaps some areas of the peritoneum (*e.g.*, the spleen) might be specifically adapted under altered conditions to allow the passage of fluid more easily than the remainder of the peritoneal lining. No evidence has been found in favor of this particular concept.

Furthermore, it is evident that, during the collection of fluid in the peritoneal cavity, under such conditions as obtained in these experi-

ments, there is a definite point at which the rate of increase becomes markedly accelerated. Just what this sudden marked increase means is not at present clear, but it is possible that there is some mechanism controlling fluid distribution which operates up to a certain point but is unable to control larger amounts and, therefore, finally breaks down and permits the peritoneal cavity to fill up. It may be that the tissues can hold a large percentage of the fluid introduced up to a certain point and that after that the conditions become reversed. This peculiar partition of fluid between tissues and serous cavities is being studied further.

CONCLUSIONS

Certain definite conclusions may be drawn from these experiments. First, neither the spleen nor the liver represent specialized areas for the transfer of fluids into the peritoneal cavity. Second, the spleen does not become edematous by the collection of fluid in any of its tissues, except the layer of mesothelial cells. Third, fluids passing through spleen and liver to the peritoneal cavity pass at least in part through the cytoplasm of the mesothelial cells.

REFERENCES

(1) CUNNINGHAM, R. S.: Studies on the spleen; (a) the rôle of the spleen in experimental hydremia; (b) reaction of splenic mesothelium to vital dyes. *Anat. Record.*, 1922, xxiii, 13–14.

(2) LOEB, L.: Edema. *Medicine*, 1923, ii, 171–348.

(3) ROWNTREE, L. G.: The water balance of the body. *Phys. Reviews*, 1922, ii, 116–170.

PLATE 1

FIG. 1. Section of spleen showing uniformly edematous mesothelial cells. × 500.

FIG. 2. Section of spleen showing edema of mesothelial cells and normal capsule. × 800.

FIG. 3. Section of spleen showing normal pulp (from same spleen as Fig. 2). × 800.

FIG. 4. Section of liver showing moderate degree of edema of liver cells. × 800.

FIG. 5. Section of liver showing marked edema of liver cells. × 800.

FIG. 6. Section of liver showing extreme degree of edema of liver cells. × 800.

Fig. 1

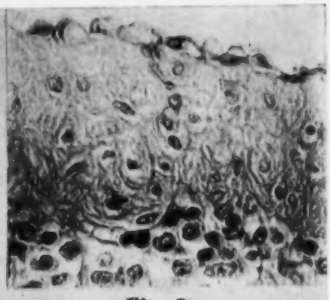

Fig. 2

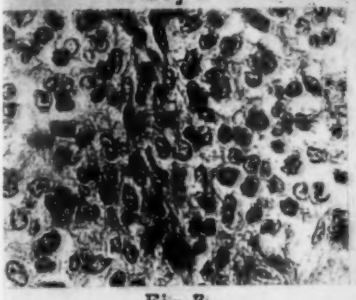

Fig. 3

Fig. 4

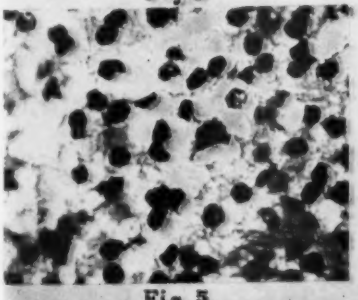

Fig. 5

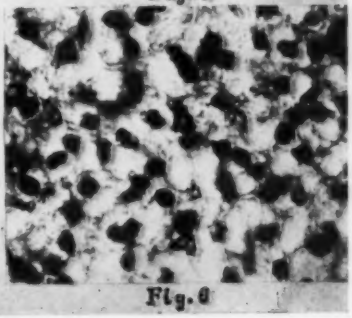

Fig. 6

UNIV. OF MICH.

A CLINICAL EVALUATION OF CHOLECYSTOGRAPHY BY THE ORAL ADMINISTRATION OF TETRA-IODOPHENOLPHTHALEIN

A SUMMARY[1,2]

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I. INTRODUCTION

The diagnosis of diseases of the gall-bladder might be said to have entered a "renaissance" since the introduction of Graham's method of studying the problem by the use of the halogenated dye, tetraiodophenolphthalein. Reports of the experiences of numerous investigators are accumulating in the literature, indicating that the dye test is giving helpful information. In the case of most diagnostic procedures, the limitations of their accuracy must be ascertained by the application of such tests to a large amount of clinical material. It is not fair, perhaps, to expect diagnostic tests to be infallible, but it is most desirable to know how high a percentage of accuracy may be expected, and to what extent such diagnostic aids may lead to errors. With this purpose in mind, then, the authors wish to present the results obtained by means of the oral method of Graham's tetraiodophenolphthalein test, in a series of 407 cases representing a variety of clinical conditions. The clinical material comes from the Wards and Out-patient Department of the Johns Hopkins Hospital; the dye films were taken by the X-Ray Department of the Johns Hopkins Hospital.

Method of administrating the dye. Twelve five-grain gelatine capsules of tetraiodophenolphthalein (fresh) were given as follows: 4 at 4 p.m., 4 at 4.30 p.m., 4 at 5 p.m. Supper at 6 p.m. No breakfast in

[1] Read before the American Gastro-Enterological Association at Atlantic City, May 3, 1927.

[2] Submitted for publication, July 19, 1927.

the morning. Dye plates were taken at 16 hours, 19 hours and 22 hours. Between plates II and III a meal containing fats was given.

Very few disagreeable symptoms resulted from taking the dye by mouth. Only one case was encountered in which dye plates could not be obtained on account of persistent vomiting.

The plates were read by both of us, in addition to the reading of the X-Ray Department of the hospital. Furthermore, they were read without any previous clinical knowledge of the case. Several cases were eliminated from our series:

	Number of cases
On account of dye in the colon obscuring the gall-bladder area	10
On account of excessive gas in the colon obscuring the plates	15
Because of the large amount of dye and gas	4
On account of poor plates	13

Three cases which showed no dye in the gall-bladder and none in the intestinal tract were excluded.

II. CLINICAL MATERIAL

All of our cases were grouped under two headings: (1) Patients operated upon, totalling 62. (2) Patients not operated upon, totalling 313. The latter group was further subdivided into (a) Patients not suspected of having any disease of the gall-bladder, judged by the usual clinical method— 142 cases; and (b) those having very definite and positive signs of gall-bladder disease—171 cases. All the patients or histories came under our personal supervision.

Our results in the cases of patients not operated upon and presenting no clinical signs of gall-bladder disease will be presented first. They are the controls. These cases were divided on the results of the Graham dye test into: (1) *Those having a normally functioning gall-bladder.* (2) *Those having an abnormally functioning gall-bladder.* The following are regarded as signs of abnormalities of function: a gall-bladder which shows little or no emptying following the meal; a very small and very thinly filled gall-bladder which may or may not empty readily; definitely mottled shadows which we are satisfied are not caused by gas, intraabdominal pressure, or material in the intestinal tract; a gall-bladder which is visualized only after the fatty meal; stones appearing in the gall-bladder with one or more of these mani-

festations. (3) *Those which do not show the faintest trace of dye in the gall-bladder.*

The analysis of this control group showed that in 69.7 per cent the gall-bladder was functioning normally; in 9.8 per cent, abnormally; and in 20.5 per cent did not fill at all. This series was carefully reviewed and it is our opinion that there were no cases of biliary disease among them. The clinical conditions comprising this group were varied, such as: visceroptosis, mucous colitis, duodenal ulcer, chronic appendicitis, nephrolithiasis, migraine, obesity, neurosis. No special member of this group of diseases predominated in the non-filling gall-bladders.

The next group to be presented includes those which we feel to be undoubted cases of gall-bladder disease (171 cases). Analysis of this series shows (1) a normally functioning gall-bladder in 34.5 per cent (2) an abnormal function in 22.9 per cent and (3) no dye in the gall-bladder in 42.5 per cent.

This group represented a series of selected patients, who with the history, symptoms and signs, from a clinical standpoint were diagnosed as having definitely diseased gall-bladders.

The most striking feature of the results in these two groups is that 20.5 per cent of the patients considered to have clinically no biliary disease showed no filling of the gall-bladder with the dye, whereas 34.5 per cent of these patients who were thought to have biliary disease showed a normally functioning gall-bladder by the Graham test. Many in this group had jaundice, gall-bladder colic or both.

III. PATIENTS OPERATED UPON

There were 62 patients in this series. Of these, 55 were subjected to operation because of clinical evidence of gall-bladder disease. The remaining 7 patients were not suspected of having biliary disease but were operated upon because of the presence of other morbid processes which needed operative treatment. All of the operation cases have been divided into three groups:

(1) Those showing a normal function (20 cases)
(2) Those showing abnormal function (6 cases)
(3) Those showing no filling with the dye (36 cases)

Analysis of these groups

1. A normally functioning gall-bladder. Of the 20 cases in this group, 11 showed a normal gall-bladder in the judgment of the surgeon at operation. The surgical reports were:

	Number of cases
Chronic appendicitis	3
Right upper quadrant adhesions	2
Duodenal ulcer	3
Exploratory operation for cancer of stomach	1
Umbilical hernia	1
Myomectomy	1

Nine cases with proven chronic cholecystitis and stones, showed a normally functioning gall-bladder. Gall-stones were found in these 9 cases. They were seen in 3 cases in the dye plates (33⅓ per cent). The Graham test was found to be correct in 11 out of 20 cases (55 per cent) and wrong in 9 cases (45 per cent). The clinical diagnosis was proven correct in 18 out of 20 cases (90 per cent). A routine microscopical examination was done on all biopsy material by the Department of Surgical Pathology and the final diagnoses reported are theirs.

The mucosa was reported as absent on microscopical examination in 3 cases that showed a normal filling with the dye.

It must be borne in mind that all these patients were submitted to operation on clinical rather than on x-ray diagnoses. Consequently, the percentage of stones found in this series is higher than would be expected, inasmuch as only the more seriously disturbed patients consented to an operation. We do not feed that the relation of stones to dye, as might seem warranted in the non-filling gall-bladder, is to be stressed.

2. Abnormally functioning gall-bladder. There were 6 cases in this series. In all a diseased gall-bladder was found at operation (100 per cent). Stones were found in 5 cases (83.3 per cent), and in 2 of these were seen in the dye plates (40 per cent). The clinical impression was correct in 4 cases (66.6 per cent) and wrong in 2 cases (33.3 per cent). The mucosa was normal in 2 cases and gone or ulcerated in the rest.

3. No dye in gall-bladder. The results of the dye test in this group of 36 cases showed a diseased gall-bladder in 30 cases (83.3 per cent) and a normal gall-bladder in 6 cases (16.7 per cent). The di-

agnoses at operation in these 6 cases were: duodenal ulcer, 2; carcinoma of the stomach, 2; chronic appendicitis, 1; myoma uteri, 1.

DISCUSSION

1. Gall-stones. Of the 9 cases in the group of patients operated upon who showed a normally functioning gall-bladder and in which on microscopical examination the gall-bladder gave definite histological evidences of cholecystitis, stones were found in all. This shows, first, that the mere presence of stones does not preclude the normal concentrating function of the gall-bladder, and second, that the association of stones and the absence of the gall-bladder mucosa will not prevent this concentration. Two cases, in each of which, on two occasions, no evidence of dye was detectable in the gall-bladder, were found, on histological examination, to be entirely normal. Hence it would appear that, although in the great majority of the cases of non-filling gall-bladders, stones were found, still the gall-bladder can give a picture of normal function with the dye when stones are present.

2. The gall-bladder mucosa. In three cases showing normal function with the dye, the gall-bladder musosa was reported as having been destroyed on histological examination, whereas in 3 cases of abnormal filling and 11 cases of non-filling it was normal. So it is seen that the gall-bladder can concentrate the dye in the total absence of mucosa, and may fail to do with this membrane normal.

3. Comparison of normal, abnormal and non-filling gall-bladders in different groups. Out of 45 cases of diseased gall-bladders normal function was diagnosed in 9 cases, an error of 20.0 per cent. In the Control Group the dye test showed no filling in 20.4 per cent. In half of these cases 2 series of x-rays were made. In the group clinically diagnosed as gall-bladder disease in which no operation was done, the gall-bladder filled normally in 34.5 per cent.

Our analysis has shown that 9 out of 20 gall-bladders giving a normal response by the dye test showed definite evidence of cholecystitis and stones. These gall-bladders were able to concentrate dye-laden bile in spite of definite disease. On the other hand, 20 per cent of gall-bladders that did not fill with the dye were deemed normal by the surgeon, and the nature of the diseased process for which the opera-

tions were performed (chronic appendicitis, adhesions, duodenal ulcer) may supply evidence to the doubtful that some of the gall-bladders were normal enough to concentrate.

The Graham test has a value as an aid in diagnosing disease of the gall-bladder. A great number of cases in this category exhibit such definite symptoms and signs that the diagnosis can be made often with certainty and correctly from these data alone. So far as our study goes, the dye test shows its greatest efficiency in this and the closely allied group of cases where the probability of gall-bladder disease is great, although some doubt may exist. Our series of cases showed the test to be correct in 83.3 per cent in this group, *i.e.*, those with non-filling gall-bladders (positive films).

The help given by the dye method in predicting the probable absence of disease of the gall-bladder is much less. As has been shown, a gall-bladder may give a normal dye test and yet be found to contain stones with an associated cholecystitis. On the other hand, a gall-bladder may show no dye and still be found to be normal. Of course, it is possible, as a great many investigators have shown since the institution of the Graham method, that the surgeon is frequently unable to make a correct diagnosis of the condition of the gall-bladder at the operating table and that after such gall-bladders have been removed, definite histological evidence of inflammation has been found in them.

CONCLUSIONS

The Graham dye test attempts to determine whether, or not, the gall-bladder is exercising its normal functions. Our studies have shown that while the method is an aid in diagnosing diseases of the gall-bladder, it has certain limitations.

1. It will not give positive assurance that a normally functioning gall-bladder is organically normal. It may give misleading information.

2. If the gall-bladder fills and empties normally with the dye, the resultant picture need not necessarily depend upon the method of administering the dye. A satisfactory film has been obtained. However, in our series of patients who were operated upon, and who showed by the dye test a normally functioning gall-bladder, 9 out of

the 20 were found to have a diseased gall-bladder. Hence it is fair to conclude that there is a probability of error in diagnosing a gall-bladder as normal, whether the dye be given intravenously or by mouth.

3. In the non-filling gall-bladders, it will not always assure the clinician that a gall-bladder is diseased, although this possibility of error is less (20 per cent).

4. A so-called delayed filling of the gall-bladder cannot be taken as evidence of early gall-bladder disease or, for that matter, of any disease. It was seen in many of our control cases, *i.e.*, they showed greater concentration in the second (19-hour) film.

5. The number of stones demonstrated by the x-ray after dye administration is decidedly greater than without it.

6. The evidence afforded by the Graham dye test must be carefully compared with the information gained from clinical studies of the cases under consideration. It is helpful but not absolute.

A STUDY OF THE LYMPHATICS OF THE GALL-BLADDER OF THE CAT[1]

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INTRODUCTION

Until recent years opinion among investigators has varied widely concerning the function of the gall-bladder. At present, however, its function has become the subject of numerous investigations and widespread discussions amongst physiologists, pathologists and clinicians. One of the interesting results of this work has been to demonstrate that the gall-bladder possesses the power of concentrating the bile which enters it. This function was first suggested by the chemical analysis of human bile by Hammersten (1905), which demonstrated a marked difference in the total dried residue obtained from equivalent fractions of hepatic and vesical bile. Rous and McMaster (1921) showed experimentally in the dog that the bile is concentrated in the gall-bladder, as evidenced by the concentration of bile-pigment when bile is passed through or stored in the gall-bladder. They found that the bile pigment may be increased as much as eight to ten times if the bile is left for a period of hours within the gall-bladder, and that the mere passage of bile through the organ is sufficient for it to become concentrated from two to four times. They postulated that this concentrating effect of the gall-bladder is due to the abstraction of water from the bile by the mucosa of the biliary reservoir. This activity of the organ is supposed to afford a means for a continuous flow of bile from the liver between periods of gastric activity when the biliary system is storing, but not delivering bile into the small intestine.

The question has been raised by recent writers whether water is the sole constituent of bile which undergoes resorption by the gall-bladder mucosa. Thus Halpert (1924), Aschoff (1924) and Sweet

[1] Received for publication July 9, 1927.

(1924) believe that other bile constituents may be removed by the action of the gall-bladder. Aschoff states that mainly water and inorganic salts are removed, although traces of bile pigment and fats also pass into the epithelium, as evidenced by their presence histologically in the mucosa. Halpert takes a similar view and describes in detail the transfer of lipoidal substances from the gall-bladder lumen into the mucosa. Sweet, from the observations of others and the work in his own laboratory, concludes that the two valvular structures at the outlet of the gall-bladder are so arranged as to permit inflow and to hinder outflow, so that under normal conditions whatever passes into the gall-bladder through the cystic duct never passes out again by the same portal. The bile entering the biliary vesicle, he believes, is eventually absorbed.

If salts and formed substances are transmitted through the gall-bladder wall, as suggested by the observations of Halpert, of Aschoff and of Sweet, a number of fascinating problems are opened up in tracing the pathway of absorption of bile constituents or experimental solutions through the biliary wall under normal and abnormal conditions. According to Rous and McMaster, absorption takes place into the lymphatics to some extent, but in greater amounts into the blood-vessels of the gall-bladder. Halpert also concludes that substances resorbed from the gall-bladder are returned to the economy of the body via the lymphatics and vascular channels. He states that he could trace lipoid substances, step by step, in their passage through the epithelial cells into the subepithelial tissue where they are found at last in phagocytic wandering cells and in the lymphatics themselves. Similarly Sweet (1924) finds that lymph from the wall of the gall-bladder gives positive chemical reactions for bile-pigments and bile-salts. Attempts to trace the pathway of absorption of a substance introduced experimentally into the gall-bladder have been made by Harer, Hargis and Van Meter (1922) and by Boyd (1923). The former stated that potassium sulphocyanide injected into the biliary vesicle can be identified chemically in the lymph-vessels almost immediately. Boyd was able to trace a solution of potassium ferrocyanide, as evidenced by subsequent precipitation as Prussian blue, in its passage through the epithelium into the stroma of the mucosal folds, but he found no indication of absorption of the foreign solution into blood-vessels or lymphatics.

FIG. 1. SEMIDIAGRAMMATIC REPRESENTATION OF THE ENTIRE GALL-BLADDER OF THE CAT, VIEWED FROM THE PERITONEAL SURFACE, SHOWING THE RELATION OF THE SUBSEROUS LYMPHATIC PLEXUS AND LARGE DRAINING TRUNKS TO THE ARTERIAL SUPPLY

Note that the subserous lymphatics and draining trunks are situated superficially to the arteries of the first and second orders. This drawing is from an actual injected specimen. Instead of two large draining trunks as represented here, several trunks may be present. The area designated "a" in this figure is shown under higher magnification in Figure 2. × 4.

The present investigator, approaching the problem of histological evidence of absorption and drainage of substances introduced into the gall-bladder, has found it advisable, before undertaking any experimental work in that direction, to study the morphology of the lymphatic plexuses in the different coats of the gall-bladder, because the accounts of the lymphatics of the gall-bladder in the literature were so incomplete. The results of these investigations form the subject of this report. Such a study is, moreover, timely because of the tendency in clinical literature to assume on quite inadequate grounds the presence of connections between the gall-bladder lymphatics and those of the liver and pancreas which are supposed to be important in the transportation of infections from one of these organs to the other. Lyon and Swalm (1925), for instance, postulate intimate lymphatic connections existing between these organs as the means for conveyance and spread of biliary tract disease.

In the current discussion of lymphatic drainage of the gall-bladder reference is usually made to the morphological investigations of Sappey (1885) and Sudler (1901), which represent our entire present-day knowledge of the anatomy of the lymph-vessels of the gall-bladder. Sappey in a plate, which is frequently reproduced, shows the lymphatics of the human liver and gall-bladder. Only the major subserous lymphatic trunks of the gall-bladder are shown, of which some ten are represented draining into four or five lymph-glands near the neck of the gall-bladder. Numerous anastomoses are also depicted between these lymph-trunks and plexuses occurring on the liver itself. Sudler's investigations concern the lymphatics of the gall-bladder of the dog, cat, pig and man. These he attempted to demonstrate by means of a Prussian blue mass injected into the subserous coat of the gall-bladder or by injection under pressure of a carmine gelatin mass into the portal vein. By these injections he succeeded in outlining a plexus of large lymph vessels in the subserosa. This plexus drained by several major trunks along the inner side of the cystic duct and ended in the mesenteris glands. The large plexus in the subserosa, he believed, received lymph not only from the gall-bladder, but from the liver as well. Moreover, by injecting carmine gelatin into the portal vein under 80 mm. of mercury for fifteen minutes, he found that it was possible to inject the lymphatics of the liver and in turn the

larger ones coursing over the gall-bladder. Sudler also traced branches from the subserous plexus which penetrated the inner layers of the gall-bladder to connect with lymphatic channels either in the subepithelial layer or in the muscular layer. This short review summarizes what is known about the lymphatics of the gall-bladder from the work of Sappey and Sudler. It leaves one in some doubt, however, as to whether they have given a totally adequate or complete picture of the pattern of the lymphatic vessels within the wall of the gall-bladder, and hence dubious as to the possible relation of lymphatics to the absorptive activity of the mucous membrane.

METHODS

Cats were employed as experimental animals and were maintained under ether anaesthesia during injection in order to obviate the disadvantages of using post-mortem material. India ink diluted with distilled water was used as the injection fluid.

The injections were made in several ways by means of a syringe bearing a 27-gauge needle. Stab injections were tried into the several layers of the gall-bladder. However, better results were obtained by introducing the point of the needle directly into a visible lymphatic of the subserous plexus. From such a point of entry, the injection fluid spread readily into the subserous plexuses and their draining trunks, as well as to some extent by retrograde passage into the lymphatics of the tunica subepithelialis (tunica propria).

Under ordinary circumstances retrograde injections could not be attained by puncture into the large draining lymphatics, because of the interference of valves. But, by following the technique devised by Lee (1922), this difficulty could be overcome. He showed that, following ligation of the thoracic duct in cats and dogs, a stasis of lymph occurred, within twenty-four hours, sufficient to cause a tremendous dilatation of the lymphatic vessels of the abdomen. Under these conditions the valves became incompetent and, taking advantage of this circumstance, he was able to make perfect retrograde injections of the lymphatics in many of the abdominal organs. By far the most complete injections of the gall-bladder were obtained by resorting to Lee's method and several cats were injected in this way.

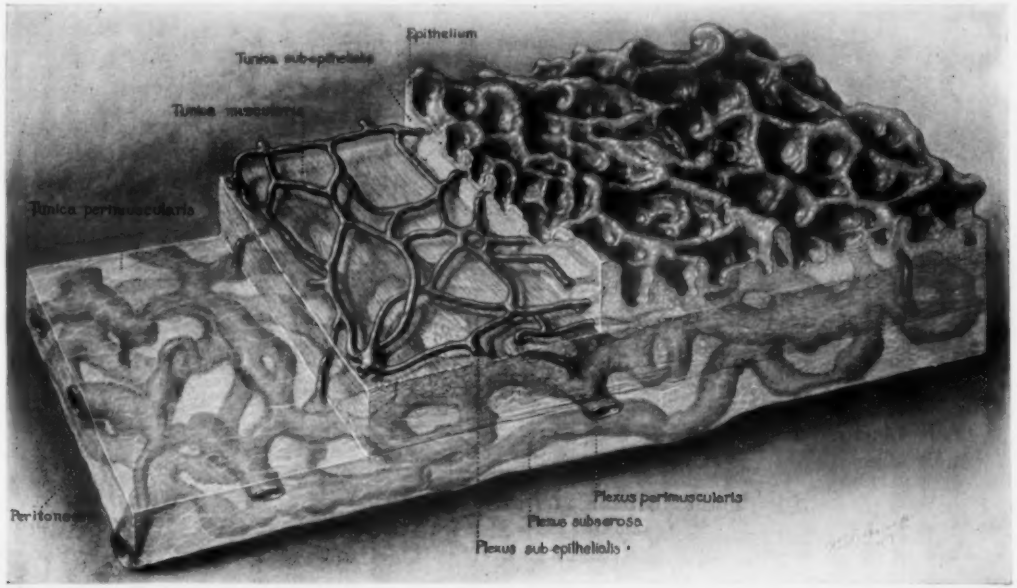

FIG. 2. SEMIDIAGRAMMATIC REPRESENTATION OF A PORTION OF THE GALL-BLADDER WALL, MODERATELY CONTRACTED, ILLUSTRATING THE LYMPHATICS AS THEY CAN BE SEEN UNDER THE GREENOUGH DISSECTING BINOCULAR MICROSCOPE IN INJECTED SPECIMENS CLEARED BY THE SPALTEHOLZ METHOD

The character of the three lymphatic plexuses is shown. The intimate relationship of the plexus of the tunica subepithelialis to the bases of the mucosal folds can be seen. The slender lymphatic vessels traversing the muscular layer and uniting the plexus of the subepithelial layer with the larger plexuses in the perimuscular layer are also pictured. × 32.

The specimens were fixed in ten per cent formalin, dehydrated in the usual manner, and cleared by the Spalteholz method. At the same time blocks were taken for section and staining. The cleared specimens were studied under the dissecting binocular microscope and cut when necessary with a safety razor blade into sections of one or two millimeters in thickness.

OBSERVATIONS

For convenience the wall of the gall-bladder of the cat may be divided, as was done by Halpert (1925), into four layers which will be termed from within outwards: (1) the mucosa, consisting of the epithelial layer and the subepithelial layer (tunica propria), (2) the muscular layer, and (3) the peri-muscular layer. On the free surface of the gall-bladder a fourth or serous layer is present.

The inner of these layers requires a brief preliminary description. The mucosa is subject to considerable variation in thickness and form in relation to the relative degree of distension of the gall-bladder at the time of fixation. When the biliary vesicle is distended, the mucosal folds, visible under the low power of the binocular in the interior of the bladder, become almost obliterated, the major folds alone persisting as fine linear elevations which divide the field under the microscope into a series of shallow, variable, polygonal depressions. On the other hand, when the gall-bladder wall is contracted, the major folds rise conspicuously into the lumen of the gall-bladder, converting its surface into curious cup-like pockets, the edges of which frequently appear to be undermined. Simultaneously secondary and tertiary folds of lesser elevation arise in the floors of these pockets, thereby contributing further to the bizarre, honey-combed appearance. These striking differences between the distended and the contracted wall account for the variable height and complexity of the folds when the organ is viewed in tissue sections. They are described here, because of the manner in which the lymphatics of the tunica subepithelialis are modified in appearance by their presence or absence.

In the cat there are three lymphatic plexuses in the wall of the gall-bladder. The innermost of these occurs in the tunica subepithelialis; the intermediate one lies in the tunica perimuscularis. These

two plexuses completely surround the gall-bladder, whereas the third or outermost plexus occurs in the subserous tissue and is therefore present only on the free surface of the organ which is unattached to the liver.

The lymphatic plexus of the tunica subepithelialis consists of a richly anastomosing network of minute lymph channels of quite regular caliber. It ramifies at varying levels, but for the most part occupies the deeper portion of the tunica subepithelialis away from the surface epithelium and adjacent to the muscular layer. In the depths of the mucosal pockets in both the contracted and distended bladder, the lymphatics come into close proximity to the epithelial layer. However, the plexus bears no obvious relation to the size and number of the folds, nor do lymph channels extend up into the folds except under conditions of extreme contraction of the surface, in which case lymphatic vessels become drawn up slightly into the exaggerated bases of the folds. In tissue sections this latter condition may simulate the appearance of finger-like projections extending up into the major folds resembling to some extent the lacteals of intestinal villi. This is the interpretation which we first placed upon the picture, but from further study of the material it has become obvious to us that such a conception is erroneous and that no free sprouts or projections occur towards the epithelial surface. No valves are present in this plexus.

From the lymphatic plexus of the tunica subepithelialis numerous straight, slender lymph-vessels arise which penetrate the muscular layer, either perpendicularly or somewhat obliquely. These vessels drain for the most part into the perimuscular plexus, but a few course beyond directly into the outermost or subserous plexus. Aside from these penetrating lymphatics the muscular layer is practically devoid of lymph vessels except for a few delicate tributaries which are received by the penetrating vessels from the intermuscular connective tissue. The lymphatic vessels of the muscular layer are devoid of valves.

The lymphatic plexus of the perimuscular coat receives the lymph from the plexus of the tunica subepithelialis. This plexus anastomoses richly and its vessels are about eight to ten times as large as those of the preceding plexus. The caliber of its channels varies

exceedingly, showing in places wide dilatations, in others, narrow constrictions. A few valves are encountered in this plexus.

The perimuscular plexus is intimately connected with the more superficially situated subserous plexus on the peritoneal surface of the gall-bladder where the latter is present. However, the former confines itself rather closely to the muscular tissue, whereas the latter lies just underneath the serosal lining. It is this subserous plexus that can often be seen in the uninjected state, with the naked eye, as irregular, refractile vessels beneath the peritoneal mesothelium. This plexus is absent over the portion of the bladder attached to the liver. Seen vertically in injected, cleared preparations under the binocular microscope, the two plexuses can be readily distinguished; the perimuscular vessels form a richly anastomosing bed, presenting irregular dilatations and constrictions of a most variable and tortuous character. Numerous channels of inconstant caliber and length course obliquely or perpendicularly to connect the two plexuses. In places, however, these interconnections may be so numerous and so short that the two plexuses appear to merge gradually into one another. Valves are met for the first time in any number in the subserous plexus, which accounts also for the difficulty in securing complete injections from one of these lymph-channels without first dilating them by the method of Lee. The vessels of the subserous plexus, while essentially of the same pattern as those of the peri-muscular coat, are in general less tortuous and irregular, with fewer anastomoses. On the whole, they resemble larger, more direct draining channels.

Large collecting ducts arise gradually by the confluence of subserous channels to form the final efferent link in the lymphatic drainage. The majority of these trunks originate from the subserous plexus over the peritoneal covered surface of the bladder and course towards the neck of the gall-bladder where they receive one or two smaller draining trunks from the peri-muscular layer of the hepatic surface of the bladder. They follow a course from the neck of the bladder towards the cystic duct where they enter the ligamentum hepato-duodenale as several stout trunks. These drain into a series of two or three lymph-nodes situated in the hepato-duodenal ligament close to the duodenum and the head of the pancreas. Near their

entry into these glands they may unite with several draining trunks from the liver.

The draining lymphatics run parallel in general to the course of the cystic artery as it passes from the neighborhood of the cystic duct to the surface of the gall-bladder. The draining trunks lie superficially to the artery, and in a fresh, uninjured specimen may be seen as glistening vessels, the contours of which bulge conspicuously from the surface of the gall-bladder. As the artery breaks up into vessels of the second order on the surface of the gall-bladder, the draining lymphatics, as they emerge from the subserous plexus, course superficially to these arterial branches. However, as the second order of arteries gives off branches of the third order, a reversal of position occurs, because the arteries continue to lie near the serosal surface, whereas the lymphatics anastomose at a slightly deeper level to form the subserous plexus.

DISCUSSION

It will be observed that we have made no mention of direct connections between the lymphatics of the liver and of the gall-bladder. Careful search has failed in the cat to reveal any intercommunication of vessels of the peri-muscular or subserous plexuses with lymphatics of the adjacent liver tissue. The only connections observed were the indirect ones established by the union of the draining trunks near their point of entry into the regional lymph-nodes, 2 to 6 cm. away from the nearest surface of the gall-bladder. Such intimate anastomoses of hepatic and biliary plexuses at the junction of the two organs, as pictured by Sappey in the human and as described by Sudler for the dog, were not to be found. In the cat, at least, in the absence of lymphatic connections, it appears impossible for a direct interchange of lymph to occur between the gall-bladder and the liver or vice versa. The only possibility of such a communication being effected would be indirectly by a retrograde flow, via the junction of the draining trunks, at some distance from the gall-bladder. Such retrograde passage would seem unlikely because of the presence of the valves in the major trunks and the subserous plexus, unless an obstruction had first rendered them incompetent.

By the use of the technique of Lee and by the Spalteholz clearing

method we have at our disposal means for injecting and studying the lymphatics of the gall-bladder in their entirety. Without these aids Sappey and Sudler were unable to study the exact relationships of the plexuses within the gall-bladder wall.

The development of the lymphatics of the gall-bladder is a subject about which nothing is known, but one which might shed some interesting light on the question of possible lymphatic connections between the liver and the biliary vesicle. The manner in which the lymphatics of the gall-bladder arise can only be surmised. The gall-bladder itself develops as a ventral, rounded subdivision of the "hepatic diverticulum" and is already differentiated in a 4 mm. human embryo (Keibel and Mall). Growing out into the mesenchyme surrounding the hepatic artery, which in turn follows the developing bile-ducts, the hepatic lymphatics follow the course of the artery. A similar growth of lymphatics along the cystic branch of the hepatic artery may be assumed as the most likely mode of growth of the biliary lymphatics.

This study was undertaken with the wider purpose in mind of investigating the absorptive function of the gall-bladder. We feel that we now know sufficient about the lymphatics in the gall-bladder wall to evaluate their rôle in such a capacity. In fact, a few experiments have been undertaken to trace the pathway of absorption of a foreign solution experimentally introduced into the gall-bladder. For this purpose, in several living, anaesthetized cats, a one and a half per cent solution of potassium ferrocyanide and iron ammonium citrate has been injected into the gall-bladder after the evacuation of several cubic centimeters of bile. After an interval of fifteen minutes to one hour the animals have been killed and the liver, with the gall-bladder and biliary tract *in situ*, has been fixed immediately in acid formalin. The outcome of these experiments has been most promising. As a result of the acid fixation, the ferrocyanide solution is precipitated as Prussian blue. In paraffin sections stained with carmalum it has been possible to trace the passage of the Prussian blue solutions through the epithelial cells of the mucosa into the stroma of the tunica subepithelialis. There the solutions seep through the tissue spaces as evidenced by the presence of precipitated Prussian blue. To what extent absorption occurs from the stroma of the

mucosal folds into the blood vascular system directly cannot be judged, but that considerable absorption takes place via the lymphatic plexus of the tunica subepithelialis, situated around the bases of the folds, is evident from the presence of Prussian blue granules in the walls and lumina of these lymphatic vessels.

SUMMARY

The wall of the gall-bladder of the cat may be divided into four layers: (1) the mucosa consisting of the epithelial layer and the subepithelial layer (tunica propria); (2) the muscular layer; and (3) the perimuscular layer. A fourth or serous layer occurs on the free surface of the gall-bladder, the part unattached to the liver.

Three lymphatic plexuses are found in the wall of the gall-bladder of the cat: 1. A plexus of delicate, anastomosing lymphatic channels, devoid of valves, in the subepithelial layer. This plexus does not extend into the stroma of the mucosal folds, nor does it send free sprouts or prolongations into these folds. It connects, however, by means of slender, straight lymph vessels, which traverse the muscular layer perpendicularly, with a second plexus in the perimuscular layer. 2. The plexus of the perimuscular layer, which consists of large anastomosing lymphatic channels of irregular contour which possess a few valves. This plexus drains through short, stout trunks into a third or subserous plexus over the free portion of the gall-bladder, but communicates directly into several draining trunks at the neck of the gall-bladder over the attached portion of the biliary vesicle. 3. The subserous plexus, consisting of large, rather straight lymphatic channels which anastomose with one another and possess numerous valves. Towards the cervical end of the gall-bladder these channels unite into a number of straight draining trunks which pursue a course in the hepato-duodenal ligament to drain into a set of lymph-nodes near the duodenum. Lymphatic trunks from the liver enter these same nodes, either independently or by first uniting with the trunks from the gall-bladder close to their entry into these lymph-glands. In the cat no direct anastomoses between lymphatics of the gall-bladder and of the adjacent liver have been found.

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REFERENCES

ASCHOFF, L. 1924. Lectures on pathology. Chap. VIII. The orthology and pathology of the extra-hepatic bile passages. P. 181, New York.

BOYD, W. 1923 Studies in gall-bladder pathology. *Brit. Jour. Surg.*, x, 337.

HALPERT, B. 1924 Neue Wege in der Gallenblasenforschung. Teil 1, *Med. Klinik*, Nr. 13, 408; Teil 2, *Ibid.*, Nr. 52, 1830.

Idem. 1925 Recent observations on the structure and function of the gall-bladder. *Anat. Rec.*, xxix, 359.

HAMMARSTEN, O. 1905 Zur Chemie der Galle. *Ergeb. d. Physiol.*, Bd. iv, 1.

HARER, W. B., HARGES, E. H., AND VAN METER, V. C. 1922 Studies of the function of the gall-bladder. *Surg. Gyn. & Obst.*, xxxiv, 307.

LEE, F. C. 1922 The establishment of collateral circulation following ligation of the thoracic duct. *Johns Hopkins Hosp. Bull.*, xxxiii, 21.

LYON, B. B., AND SWALM, W. A. 1925 The therapeutic value of non-surgical drainage of the biliary tract. *Jour. Am. Med. Assn.*, **85**: 1541.

ROUS, P., AND MCMASTER, P. D. 1921 The concentrating activity of the gall-bladder. *Jour. Exper. Med.*, xxxiv, 47.

ROUS, P., BROUN, G. O., AND MCMASTER, P. D. 1923 Studies on the total bile. *Jour. Exper. Med.*, xxxvii, 685.

SAPPEY, PH. C. 1885 Vaisseaux lymphatiques considérés chez l'homme et les vertébrés. Paris.

SUDLER, M. T. 1901 The architecture of the gall-bladder. *Johns Hopkins Hosp. Bull.*, xii, 126.

SWEET, J. E. 1924 The gall-bladder: its past, present and future. *Internat. Clinics*, i, 34th. Ser., p. 187.

BOOK REVIEW

International Clinics. By HENRY W. CATTELL. Vol. I. Thirty-seventh Series. March, 1927. 304 pp. $3.00. (*J. B. Lippincott Co., Philadelphia, 1927.*)

The current issue of this series of International Clinics contains three papers worthy of note. The article by Harbitz presents pathological findings in several unusual forms of arteritis, including periarteritis nodosa, and makes a plea for more careful study of vascular disease. Van Wely points out the danger of inducing bundle-branch block, and even complete heart block, with quinidine in the treatment of certain cardiac irregularities. Davidson, in a very interesting communication, seems to have proved by the citation of cases that certain types of cardio-vascular disease are capable of producing symptoms and findings that might easily be mistaken for better known, chronic organic conditions, such as cancer or ulcer of the stomach.

In addition to these articles, it is worth while to call attention to the patient with congenital urethral valve, demonstrated in Dr. Lewis' surgical clinic, and successfully treated by removal of the obstructing tissue. The writer had the opportunity to follow this patient rather closely, and was impressed with the fact that it is obligatory, in any case of uremia, regardless of age, to consider obstruction as a possible underlying cause.

B. W. L.